NT TO JONATHAN - MONDAY, MAY 24, 1993

lycerol stocks - (1 ml in 15% glycerol, log phase culture of DH5alphaF' containing plasmid)

pVR2 - replicon, VEE cDNA clone downstream of a T7 promoter with the structural genes deleted, 26S promoter followed by, in order, a unique ClaI site, the complete 3'-untranslated region, $(A)_{21}$, and a unique NotI site in a pBR322-derived vector with *bla* and an origin of replication.

pVR2HA 41 - replicon with the complete influenza HA gene inserted in the coding orientation (pVR2 with the 26S promoter followed by, in order, a ClaI site, the HA gene inserted into the HindIII site of the multiple cloning region of the shuttle vector, and a second ClaI site. (Multiple cloning region of shuttle vector is shown below.)

pVR2HA 71 - same as pVR2HA 41 with HA gene in noncoding orientation.

pUC11826SP21-4 - shuttle vector, VEE structural gene region with second 26S promoter inserted immediately downstream of the C-terminus of E1 followed by a multiple cloning site flanked by ClaI sites, the complete 3'-untranslated region, and a unique NotI site (diagram shown below).

iprep DNA - 10 ul (approximately 2 ug of plasmid DNA in TE buffer)

pVR2, pVR2HA 41, pVR2HA 71

RNA transcripts - 9 ul (approximately 7 ug RNA made from 0.7 ug DNA template)

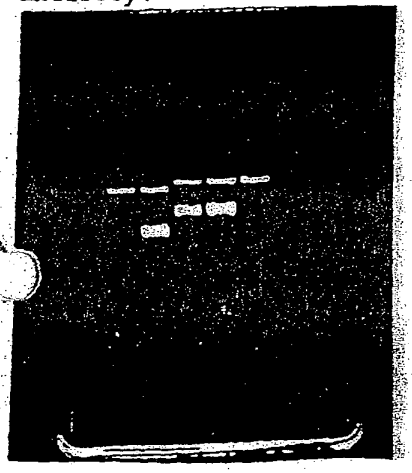
pVR2, pVR2HA 41, pVR2A 71

Monoclonal antibody is being sent separately FED. EX. on wet ice.

Mab 37-85. Staudt and Gerhard. 1983. J. Exp. Med. 157:687.
I used this with a Vectastain HRP protocol, blocking with 7.5% BSA, undiluted onto BHK cell monolayers from a 16-well chamber slide. It probably can be diluted somewhat. Getting more of this antibody would take considerable effort (getting the cell line, etc.), so you may want to consider another reporter gene, or finding another source for an anti-influenza PR8 antibody.

Good luck!

James



Gel of RNA txn rxns
1. pVR2 template DNA
2. " Txn
3. pVR2HA41 Txn
4. pVR2HA71 Txn
5. pVR2HA71 template DNA

EXHIBIT F